Live cell monitoring of hiPSC generation and differentiation using differential expression of endogenous microRNAs.

Journal: PLoS One

Publication Year: 2010

Authors: Masakazu Kamata, Min Liang, Shirley Liu, Yoshiko Nagaoka, Irvin S Y Chen

PubMed link: 20676373

Funding Grants: Genetic modification of the human genome to resist HIV-1 infection and/or disease progression, HPSC based therapy for HIV disease using RNAi to CCR5., Stem Cell Therapy for AIDS- Disease Team Planning

Public Summary:
Human induced pluripotent stem cells (hiPSCs) provide new possibilities for regenerative therapies. In order for this potential to be achieved, it is critical to efficiently monitor the differentiation of these hiPSCs into specific lineages. Here, we describe a lentiviral reporter vector sensitive to specific microRNAs (miRNA) to show that a single vector bearing multiple miRNA target sequences conjugated to different reporters can be used to monitor hiPSC formation and subsequent differentiation from human fetal fibroblasts (HFFs).

Scientific Abstract:
Human induced pluripotent stem cells (hiPSCs) provide new possibilities for regenerative therapies. In order for this potential to be achieved, it is critical to efficiently monitor the differentiation of these hiPSCs into specific lineages. Here, we describe a lentiviral reporter vector sensitive to specific microRNAs (miRNA) to show that a single vector bearing multiple miRNA target sequences conjugated to different reporters can be used to monitor hiPSC formation and subsequent differentiation from human fetal fibroblasts (HFFs). The reporter vector encodes EGFP conjugated to the targets of human embryonic stem cell (hESC) specific miRNAs (miR-302a and miR-302d) and mCherry conjugated to the targets of differentiated cells specific miRNAs (miR-142-3p, miR-155, and miR-223). The vector was used to track reprogramming of HFF to iPSC. HFFs co-transduced with this reporter vector and vectors encoding 4 reprogramming factors (OCT4, SOX2, KLF4 and cMYC) were mostly positive for EGFP (67%) at an early stage of hiPSC formation. EGFP expression gradually disappeared and mCherry expression increased indicating less miRNAs specific to differentiated cells and expression of miRNAs specific to hESCs. Upon differentiation of the hiPSC into embryoid bodies, a large fraction of these hiPSCs regained EGFP expression and some of those cells became single positive for EGFP. Further differentiation into neural lineages showed distinct structures demarcated by either EGFP or mCherry expression. These findings demonstrate that a miRNA dependent reporter vector can be a useful tool to monitor living cells during reprogramming of hiPSC and subsequent differentiation to lineage specific cells.